Probiotic Potential of Fermented Yam Wash Water and Unprocessed Milk: Lactobacillus Isolation and Characterization

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Abstract**—** *This study investigated the fermented yam wash water and raw milk for their probiotic potentials through detailed isolation and characterization of lactobacillus. There were a total of eleven different types of lactobacilli isolated by traditional methods of fermentation. Their morphological and biochemical characteristics were well studied. A number of microbiological methods such as Gram staining, application of various biochemical tests and microscopic examinations were performed to accurately identify and characterize bacteria. Results showed concordant results in species of lactobacillus with variant biochemical profiles, indicating the rich microbial potential of indigenous fermentation processes. Similarly, enzymatic capabilities demonstrated variability, hence giving variable probiotic functionality. All the eleven samples were identified to belong to Lactobacillus spp. based on striking differences from the oxidase and catalase reactions. This study serves as formidable evidence in the application of locally fermented substrates in probiotic interventions, particularly in animal nutrition. These findings have bearing on the understanding of indigenous fermentation techniques and further development of probiotics in a sustainable manner within agricultural systems.*

Keywords**— *Fermentation techniques, microbiological methods, lactobacillus spp, unprocessed milk, yam wash water.***

I. INTRODUCTION

Probiotics have emerged as one of the leading areas of research into animal nutrition and health, presenting promising alternatives to traditional antibiotic interventions in livestock productions (Cameron and McAllister, 2019). Lactobacillus species have, therefore, taken centre stage for their immense potentials in enhancing the health of animals and improving gut microbiota for enhancing overall performance in poultry production (Shivajyothi and Krishna, 2020). With an increased world demand for animal protein that is safe and produced in a sustainable manner, impetus has arisen for researchers to find new sources of probiotics which can be developed locally and inexpensively.

Fermentation is one classic biotechnological process yielding useful micro-organisms (Maicas, 2023). Products of fermentation had proven their great potential to be used in agriculture for the development of probiotic cultures containing huge nutritional as well as health-enhancing traits (Dahiya and Nigam, 2022). Traditional techniques of fermenting substrates available at local levels, including yam and milk, have lately started offering an exciting opportunity for developing probiotics for the tropics (Ilango and Antony, 2021).

Considering nutritional value and the prospect of being a good fermentation substrate, yam-one of the staple crops in most African countries-has been underutilized over the years for microbial studies. In this regard, Price *et al.* (2017) showed that yam has good intrinsic microbiological qualities that may support microbial life and related metabolic activity. On the other side, raw milk is a complex substrate with high levels of a variety of nutrients that could make growth and proliferation of efficient bacterial strains easy (Medvedova *et al.,* 2020).

Poultry production in developing countries seriously suffers in respect of animal health and production due to high antibiotic resistance and general poor veterinary interventions (Mottet and Tempio, 2017). One promising strategy is the use of probiotic administration that could improve feed conversion ratios and enhance the status of immune responses and reduce pathogenic loads within livestock systems (Mia *et al.,* 2024). As Ringø *et al.* (2020) has suggested, locally derived probiotics could be one option. However, local probiotic development is at its infancy; therefore, serious research on indigenous microbial resources needs to be done.

Literatures vary with a number reporting efficacy on Lactobacillus strains that perform better in broiler chickens; specific Lactobacillus isolates selected have been shown to exert positive modulation of gut microbiota, enhance nutrient absorption, and elicit immune responses (Zhao *et al.,* 2022). Locally isolated lactobacillus strains should perhaps be characterized and utilized for regional probiotic interventions.

These are gaps that this present research has attempted to fill by undertaking an intensive study on the probiotic potentials of fermented yam wash water and raw milk. Consequently, this research attempts to isolate, identify and describe the species from Lactobacillus in these local substrates and assess their probiotic potentials for broiler chicken nutrition. The study contributes to a better understanding of traditional fermentation techniques and their microbiological end products as one avenue toward sustainable probiotic development in agriculture. Hence, this research is designed to isolate and identify Lactobacillus species from fermented yam wash water and raw milk, describe the biochemical and morphological characteristics, and further explore the potentials of probiotic functions through the assay of its efficacy in improving performance and health parameters in broilers.

This study marries traditional fermentation knowledge with modern microbiological techniques for new frontiers in the development of probiotics. Besides the scientific merit, it would provide a potent, affordable, and locally made probiotic means of enhancing the productivity and health of livestock to local farmers and other crop producers.

II. MATERIALS AND METHODS

2.1 Experimental Site:

The study was conducted at the Poultry Unit of the Teaching and Research Farm, Department of Animal Science and Technology, Faculty of Agriculture, Nnamdi Azikiwe University, Awka campus, Anambra State, Nigeria. The location is situated at Latitude 6°12'25"N and Longitude 7°04'04"E, with an average altitude of 9 meters above sea level.

2.2 Collection and Preparation of Experimental Materials:

2.2.1 Raw Materials:

Unprocessed cow milk was sourced from the Amansea Abattoir, while water yam tubers were purchased from Oye Agu Market in Abagana, Njikoka Local Government Area of Anambra State.

2.2.2 Fermentation Process:

Fermentation of yam wash water and milk was carried out at No. 2 Ezingwu Street, Umuokpu Awka, using the method described by Jones (2024). The yams were washed, peeled, diced and washed in the ratio of 1:1.5kg yam to 1.5 litres of water. The first two cloudy rinse waters were collected in a clean white jar and covered with muslin cloth fixed with a rubber band. The jar was then kept on a shelf, undisturbed, to ferment. After five days, a sour odour deduced lactobacillus multiplication.

The floating scum was skimmed off while the pale-yellow layer was decanted into the fresh unprocessed cow milk in the ratio of 10: 1 parts milk to one part yam wash water. It was left to ferment for five more days to separate the liquid and solid forms.

2.3 Isolation and Identification of Lactobacillus:

2.3.1 Sample Collection:

Eleven samples of fermented yam wash water and unprocessed milk were collected for laboratory analysis.

2.3.2 Identification Methodology:

Lactobacillus species was identified according to the methods described in Bergey's Manual of Systematic Bacteriology (Sneath *et al.,* 1986). Identification involved the:

1) Physical and microscopic observation of colony morphologies on selective media.
2) Gram staining to observe cell morphology.
3) Confirmation of Gram-positive, catalase-negative, non-spore-forming, rod-shaped isolates.

2.3.3 Biochemical Characterization:

Final identification employed the following classical microbiological tests: Gram staining, catalase and oxidase tests, motility test, indole production test, growth at 15°C and carbohydrate fermentation tests for: Arabinose, Fructose, Galactose, Lactose, Mannitol, Salicin, Sucrose and Trehalose

2.4 Probiotic Mixture Preparation:

2.4.1 Preparation of Bacterial Culture:

1) Inoculation of the isolates in MRS broth was done and incubated for 48 h at 37°C.
2) Tubes were centrifuged at 5000 rpm for 5 min.
3) Cells were harvested and washed three times with Phosphate Buffered Solution (PBS).
4) Pellets were dissolved in PBS and again centrifuged at 10,000 rpm for 5 min.
5) Added 300 μL of 30% glycerol in each tube.
6) Samples were stored at -80°C.

Strains were evaluated for growth and stability by viable cell count after one week storage under refrigeration in a liquid fermentation medium.

2.4.2 Microbiology Laboratory:

A sample of the fermented mixture was then taken to the Microbiology Laboratory, School of Pharmaceutical Studies, Nnamdi Azikiwe University, Agulu campus for the species identification of lactobacillus.

2.5 Statistical Analysis:

Data collected during the experiment were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD) with a 2 × 4 factorial arrangement in CRD. Data analysis was carried out using the SPSS statistical package, version 20. Means showing significant difference were separated using the LSD test at 5%. However, systematic comparison of the treatment effects through time allows any statistically significant differences among the experimental groups to be determined and provides rigorous statistical validation of the research findings.

III. RESULTS AND DISCUSSION

TABLE 1

RESULT OF FINDINGS ON LACTOBACILLUS CONTENT OF THE YAM WASH WATER AND MILK MIXTURE

Sample code	Colonial morphology / characteristic	Gram character	Microscopic feature	Biochemical / confirmatory test		Probable organism
				Oxidase	Catalase	
1	Milky, mucoid colonies	+ve	Oval purple rods	+ve	+ve	*Lactobacillus spp*
2	Milky, mucoid colonies	+ve	Oval purple rods	-Ve	-Ve	*Lactobacillus spp*
3	Milky, mucoid colonies	+ve	Oval purple rods	-Ve	-Ve	*Lactobacillus spp*
4	Milky-brown, mucoid colonies	+ve	Oval purple rods	+Ve	+Ve	*Lactobacillus spp*
5	Milky-brown, mucoid colonies	+ve	Slender Oval purple rods	+Ve	+Ve	*Lactobacillus spp*
6	Milky, mucoid colonies	+ve	Oval purple rods	-Ve	-Ve	*Lactobacillus spp*
7	Milky, mucoid colonies	+ve	Oval purple rods	-Ve	-Ve	*Lactobacillus spp*
8	Milky, mucoid colonies	+ve	Oval purple rods	-Ve	-Ve	*Lactobacillus spp*
9	Milky, mucoid colonies	+ve	Oval purple rods	-Ve	-Ve	*Lactobacillus spp*
10	Milky, mucoid colonies	+ve	Oval purple rods	-Ve	-Ve	*Lactobacillus spp*
11	Milky-brown, mucoid colonies	+ve	Slender Oval purple rods	+Ve	+Ve	*Lactobacillus spp*

3.1 Lactobacillus Characterization and Identification:

The fermentation of the yam wash water-milk mixture presented a complete spectrum of lactobacillus isolates, mapping the tremendous microbial diversity traditionally encapsulated in any fermentation process. A total of eleven bacterial isolates of dissimilar types were obtained showing different morphology and biochemical characteristic features typical of lactobacillus species presented on Table 1.

It was observed that colony morphology for the samples was fairly similar, characterized mainly by milky and mucoid colonies typical for lactobacillus organisms. The relatively consistent microbial morphology could indicate a steady state in the microbial fermentation conditions and, therefore, good substrate conditions by which lactobacillus organisms could flourish. The presence of milky and mucoid colonies agrees with earlier observations on the morphological features of lactobacillus cultures in fermented broths as opined by Gutiérrez *et al.* (2016) and in much older studies as seen in Norris *et al.* (1954).

Microscopical observation gave a really interesting insight into the morphological diversities among the isolates. All the samples (except sample 5 and 11), had been in the form of oval purple rods. Sample 5 and 11 had slender oval purple rod morphology. Such minor variations in morphology underscore the complex heterogeneity within lactobacillus populations, pointing out the possibility for subspecies or even strain level differences that could influence functionalities as a probiotic.

Gram staining showed positive results for all the eleven isolates, thus re-affirming that the isolate was a Gram-positive bacterium. This is a primary means of characterization of lactobacillus and is important in microbiological taxonomy by Zheng *et al* (2020). The consistency in Gram-positive Gram reaction further verifies the efficiency of the method of isolation and identification adopted in this work.

Confirmatory biochemical tests indicated the interesting trend in oxidase and catalase reactions, where six isolates (samples 2, 3, 6, 7, 8, 9, and 10) were negative in respect to both oxidase and catalase, while five isolates (samples 1, 4, 5, and 11) gave positive results. These so-called biochemical differences also indicate their metabolic diversity, which may substantially be responsible for different probiotic effects and functional properties.

However, the most striking observation has been the consistent identification of all these isolates as *Lactobacillus spp*; thus, showing the enormous microbial potential of the fermented yam wash water and milk mixture. This substantiates the hypothesis that traditional fermentation techniques are most conducive to developing a diverse population of potentially probiotic bacterial strains (Ilango & Antony, 2021).

3.2 Probiotics Application and Implication:

The observed microbial diversity presents compelling evidence for the probiotic potential of locally fermented substrates. The consistent prevalence of lactobacillus strains bearing various biochemical profiles may present a rich repository of putative beneficial microbes. Previous studies have shown that such diverse lactobacillus populations are capable of showing multiple health benefits in animal nutrition by modulating gut microbiota, enhancing immune responses, and possibly inhibiting pathogens in several cases (Rastogi and Singh, 2022).

This biochemical heterogeneity, as represented by the oxidase and catalase reactions, might indicate a difference in metabolic capability among the isolates. Accordingly, strains with different enzymatic profiles may offer different modes of probiotic action, which could involve the improvement of nutrient metabolism, competitive exclusion of pathogens, or some other immunomodulatory function(s) (Plaza-Diaz *et al.,* 2019).

3.3 Comparative Setting and Significance of Research:

These findings contribute significantly to the few studies into indigenous fermentation techniques as sources of probiotic microorganisms. The present study has further demonstrated the ability of traditional fermentation techniques using locally available substrates, such as yam and milk, to generate a rich, probably highly valuable microbial ecology. Consistency in morphological and biochemical characters in isolates outlines a very consistent and stable microbial microenvironment throughout fermentation-a very important aspect necessary for the development of dependable probiotic interventions, especially in agricultural settings that have high demands on consistent microbial performances.

While the current study provides comprehensive insights into the characterization of lactobacillus, further studies should focus on; determination and complete genetic characterization of identified strains, deep assessment of the individual probiotic strain potential, evaluation of strain-specific performance in animal nutrition models. Results obtained highlight the prospects of indigenous fermentation techniques being a very valuable approach toward the development of probiotics-a promising avenue to ensure sustainable and locally derived microbial solutions in agricultural systems.

IV. CONCLUSION AND RECOMMENDATIONS

This in-depth study of the probiotic potential of fermented yam wash water and unprocessed milk has provided very important insights into the microbial diversity and potential of indigenous fermentation techniques. The present study was able to isolate and characterize eleven distinct strains of Lactobacillus, showing high microbial richness inherent in traditional fermentation processes. These findings are of huge importance to locally sourced substrates for generating valuable probiotic microorganisms with diverse biochemical and morphological characteristics.

The constant presence of species of the genus Lactobacillus was proven for all isolates, with strong variability expressed in biochemical profiles and microscopic characteristics. These variations might mean differential functionality of probiotics and open exciting possibilities for developing specific probiotic interventions in animal nutrition. This further underlines the intricate microbial ecosystem formed by traditional fermentation processes through the constantly Gram-positive nature and diversity in enzymatic abilities of the isolated strains. These will form the basis for more tailored genetic characterization of strains found to be Lactobacillus, complete investigation into the probiotic potential of each strain, and deep assessment of their specific performance in animal nutrition models. Further research into mechanisms of probiotic action and possible immunomodulating function of these strains with standardized protocols on how to select and apply these probiotics in agriculture is therefore warranted.

These findings justify the expectation of developing locally sourced, inexpensive probiotic products. Crossing traditional fermentation knowledge with new techniques from microbiology can truly let the researchers open entirely new venues for sustainable innovation in agriculture. The presented research puts an emphasis on the critical value of indigenous fermentation techniques, which are of great importance in generating high-value microbial resources that probably enable improvement in productivity and health of livestock.

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